

DP Barcode: 357928

MRID No: 47555301

**DATA EVALUATION RECORD
FISH ACUTE TOXICITY MITIGATED BY HUMIC ACID
GUIDELINE OPPTS 850.1085**

CHEMICAL: Didecyldimethylammoniumchloride (DDAC)

PC Code No.: 069149

TEST MATERIAL: Didecyldimethylammoniumchloride (DDAC),
Batch #B-1889

Purity: 80.5%

CITATION:

Author: A.E. Putt, A.E.

Date: 1994

Title: Didecyldimethylammoniumchloride (DDAC) – Static Acute Toxicity to Fathead
Minnow (*Pimephales promelas*) With and Without the Presence of Humic Acid

Laboratory: Springborn Laboratories, Inc., Wareham, Massachusetts

Sponsor: Lonza Inc., Fair Lawn, New Jersey

Study Report ID: 11696.0893.6111.101

Laboratory Report ID: 94-1-5122

MRID No.: 47555301

REVIEWED BY: William Erickson, Biologist

Signature:

Date:

APPROVED BY: Norm Cook, Branch Chief

Signature:

Date:

STUDY PARAMETERS:

Scientific Name of Test Organism: *Pimephales promelas*

Age of Test Organism: Juvenile (presumed based on reported weight and length)

Definitive Test Duration: 96 hours

Study Method: Static Renewal

Type of Concentrations: Mean measured

RESULTS SYNOPSIS:

DDAC (clean dilution water):

96-hr LC₅₀: 0.19 mg a.i./L 95% C.I.: 0.16 to 0.27 mg a.i./L NOEC: 0.092 mg a.i./L

DDAC with 10 mg/L of Humic Acid:

96-hr LC₅₀: 0.77 mg a.i./L 95% C.I.: 0.65 to 1.0 mg a.i./L NOEC: 0.40 mg a.i./L

DAC with 20 mg/L of Humic Acid:96-hr LC₅₀: 1.2 mg a.i./L 95% C.I.: 0.94 to 1.6 mg a.i./L NOEC: 0.94 mg a.i./L**ADEQUACY OF THE STUDY:****Classification:** Core**Rationale:** n/a**GUIDELINE DEVIATIONS:**

The following guideline deviations were based on EPA OPPTS Guideline 850.1085:

- Test fish not acclimated in accordance with guidelines.
- Abnormal behavior and observations of compound solubility not reported.
- Apparatus for straining particulate matter not reported.
- Cleaning of test system prior to initiation of test not reported.
- DO concentration fell below guideline level during test

MATERIALS AND METHODS:**Test Organisms**

Guideline Criteria	Reported Information
Species <ul style="list-style-type: none"> ▪ Preferred freshwater species: bluegill sunfish (<i>Lepomis macrochirus</i>) or rainbow trout (<i>Oncorhynchus mykiss</i>) ▪ Preferred saltwater species: Atlantic silverside (<i>Menidia menidia</i>) or Sheepshead minnow (<i>Cyprinodon variegatus</i>) 	<ul style="list-style-type: none"> ▪ Fathead minnow (<i>Pimephales promelas</i>)
Weight <ul style="list-style-type: none"> ▪ Juvenile fish < 3.0 g 	<ul style="list-style-type: none"> ▪ Mean = 0.63 g ▪ Range = 0.39 to 0.91 g
Length <ul style="list-style-type: none"> ▪ Longest not > 2x shortest 	<ul style="list-style-type: none"> ▪ Mean = 41 mm
Supplier	<ul style="list-style-type: none"> ▪ Springborn Laboratories culture facilities
All fish from same source and population?	<ul style="list-style-type: none"> ▪ Not reported
Fish used in previous tests?	<ul style="list-style-type: none"> ▪ Not reported
If wild fish used, quarantined 7 days before acclimation?	<ul style="list-style-type: none"> ▪ Not applicable
Signs of stress or injury?	<ul style="list-style-type: none"> ▪ Not reported

Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> <ul style="list-style-type: none"> Minimum 12 days (14 days recommended) Minimum 7 days in test dilution water 	<ul style="list-style-type: none"> 14 days
<u>Holding Water</u> <ul style="list-style-type: none"> Same source as test dilution water (if not, acclimation to dilution water done gradually over 48 hr period) 	<ul style="list-style-type: none"> Holding water was not from same source as test dilution water, but has similar characteristics.
<u>Disease Treatment</u> <ul style="list-style-type: none"> No treatments within 48 hrs of test initiation or during test 	<ul style="list-style-type: none"> Not reported
<u>Feeding</u> <ul style="list-style-type: none"> No feeding within 48 hrs of test initiation. Feed daily prior to this period. 	<ul style="list-style-type: none"> Not fed within 48 hours of test initiation or during test, but fed daily during the holding period.
<u>Pretest Mortality</u> <ul style="list-style-type: none"> < 5% during acclimation; reject entire batch if > 10%. 	<ul style="list-style-type: none"> No mortality observed during 48-hour period prior to test initiation.
<u>Water Temperature</u> <ul style="list-style-type: none"> Temperature changes should not exceed 3°C per day Hold fish minimum 7 days at test temperature prior to testing 	<ul style="list-style-type: none"> Temperature ranged from 24 to 26 °C during 14-day holding period.
<u>Background</u> <ul style="list-style-type: none"> During final 48 hrs, colors and light intensities similar to testing area 	<ul style="list-style-type: none"> Held in stainless steel tank under a photoperiod of 16 hours light and 8 hours darkness (photoperiod same as testing area).

Test System

Guideline Criteria	Reported Information
<u>Dilution Water</u> <ul style="list-style-type: none"> Clean surface or ground water, reconstituted water, or dechlorinated tap water (if the test fish will survive in it for the duration of the holding, acclimating and testing periods without showing signs of stress) Chemical analysis performed and maximum concentrations not exceeded (see guideline) 	<ul style="list-style-type: none"> Soft water reconstituted from deionized water according to recommended procedures. Chemical analysis performed.

Guideline Criteria	Reported Information
<u>Solutions</u> <ul style="list-style-type: none"> Distilled water used to make stock solutions of test substances. If stock volume > 10% of test solution volume, dilution water used. 	<ul style="list-style-type: none"> Distilled water used to make stock solutions.
<u>Water Temperature</u> <ul style="list-style-type: none"> 10 or 12 ± 2°C for cold water species (see guideline) 22 or 23 ± 2°C for warm water species (see guideline) Vary no more than 1°C in any 24-hr period Record in all replicates at beginning of test and every 24 hrs; record hourly in one replicate. 	<ul style="list-style-type: none"> Measured in each replicate at the beginning of test and every 24 hrs. DDAC (clean dilution water): 22 to 24 °C. DDAC with 10 mg/L of Humic Acid: 21 to 23 °C. DDAC with 20mg/L of Humic Acid: 22 to 23 °C.
<u>pH</u> <ul style="list-style-type: none"> > 6.0 and < 8.0 for freshwater testing > 7.5 and < 8.5 for marine testing Measured in each replicate at beginning of test and every 24 hrs 	<ul style="list-style-type: none"> Measured in each replicate at the beginning of test and every 24 hrs. DDAC (clean dilution water): Ranged from 6.7 to 8.0. DDAC with 10 mg/L of Humic Acid: Ranged from 7.1 to 8.4. DDAC with 20mg/L of Humic Acid: Ranged from 6.8 to 7.8.
<u>Dissolved Oxygen</u> <ul style="list-style-type: none"> Static: should be maintained above 5.5 mg/L for rainbow trout and above 4.5 mg/L for bluegill and fathead minnow Flow-through: > 75% saturation at all times Measured in each replicate at beginning of test and every 24 hrs 	<ul style="list-style-type: none"> Measured in each replicate at the beginning of test and every 24 hrs. DDAC (clean dilution water): Ranged from 4.1 mg/L to 9.6 mg/L. DDAC with 10 mg/L of Humic Acid: Ranged from 0.2 mg/L to 9.0 mg/L. DDAC with 20mg/L of Humic Acid: Ranged from 1.6 mg/L to 9.0 mg/L..
<u>Total Hardness</u> <ul style="list-style-type: none"> 40 to 180 mg/L as CaCO₃ (freshwater species) Measured at beginning of each test 	<ul style="list-style-type: none"> DDAC (clean dilution water): 40-42 mg/L. DDAC with 10 mg/L of Humic Acid: 36 mg/L. DDAC with 20mg/L of Humic Acid: 38-40 mg/L .
<u>Salinity</u> <ul style="list-style-type: none"> 20 ± 5ppt (estuarine species) Measured at beginning of each test and, for flow-through tests, on day 4, and if extended days 7 and 14 	<ul style="list-style-type: none"> n/a

Guideline Criteria	Reported Information
<u>Test Aquaria/Equipment</u> <ul style="list-style-type: none"> Material: Glass, stainless steel, nylon screen or perfluorocarbon plastic (e.g., Teflon®) Test chambers loosely covered 	<ul style="list-style-type: none"> 18.9-L glass aquaria
<u>Aeration</u> <ul style="list-style-type: none"> Static systems only if < 60% saturation; if aeration used test concentrations measured. No aeration in flow-through tests 	<ul style="list-style-type: none"> Not aerated
<u>Type of Dilution System</u> <ul style="list-style-type: none"> Must provide reproducible supply of toxicant 	<ul style="list-style-type: none"> Replicate test solutions were prepared individually by adding the appropriate volume of the 10 mg a.i./mL DDAC stock solution to 15 L of dilution water in each test aquaria.
<u>Flow Rate</u> <ul style="list-style-type: none"> Consistent flow rate of 6-10 vol/24 hours Measured at beginning and end of each test No more than a factor of 10 variation between replicates 	<ul style="list-style-type: none"> Not applicable
<u>Biomass Loading Rate</u> <ul style="list-style-type: none"> Static/Static-renewal: ≤ 0.8 g FWF/L Flow-through: ≤ 0.5 g FWF/L 	<ul style="list-style-type: none"> 0.42 g/L
<u>Photoperiod</u> <ul style="list-style-type: none"> Range from 12D/12N to 16D/8N, with 15 min transition period Intensity 30 to 100 lm at water surface 	<ul style="list-style-type: none"> 16D/8N Light intensity for DDAC (clean dilution water) ranged from 20 to 70 footcandles Light intensity for DDAC with 10 mg/L of Humic Acid) was 40 footcandles Light intensity for DDAC with 20mg/L of Humic Acid) ranged from 55 to 70 footcandles
<u>Solvents</u> <ul style="list-style-type: none"> Not to exceed 0.5 ml/L for static or static-renewal tests or 0.1 ml/L for flow-through tests Preferred solvents dimethyl formamide, triethylene glycol, methanol, acetone, or ethanol 	<ul style="list-style-type: none"> Not reported

Test Design

Guideline Criteria	Reported Information
<p><u>Range-Finding Test</u></p> <ul style="list-style-type: none"> If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test required 	<ul style="list-style-type: none"> Range-finding test conducted at Springborn. 10 fathead minnow per treatment level were exposed for 96 hours under static conditions to nominal concentrations of DDAC ranging from 0.10 to 5.0 mg a.i./L. After 24 hours 100% mortality observed at exposure concentrations greater than 0.50 mg a.i./L. After 96 hours no mortality or sublethal effects observed at exposure to 0.10 mg a.i./L.
<p><u>Test Concentrations</u></p> <ul style="list-style-type: none"> Minimum of control and 5 concentrations in geometric series Concentrations 50 to 120% greater than next lowest concentration No more than 25% variation between test concentrations within same treatment Concentrations selected to produce NOEC and, preferably, at least 2 partial mortalities (> and < 50%) after 96 hrs Measured concentrations required if test chemical unstable or flow-through system, and must remain at least 80% of nominal concentrations 	<ul style="list-style-type: none"> 2 controls: clean dilution water and humic acid control. 5 concentrations for DDAC in clean dilution water: 0.054, 0.092, 0.16, 0.27 and 0.46 mg a.i./L. 5 concentrations for DDAC with 10 mg/L of Humic Acid: 0.40, 0.65, 1.0, 1.6 and 2.6 mg a.i./L. 5 concentrations for DDAC with 20 mg/L of Humic Acid: 0.35, 0.51, 0.94, 1.6 and 2.5 mg a.i./L.
<p><u>Concentration Analysis</u></p> <ul style="list-style-type: none"> Performed at test initiation and every 48 hrs Static: each replicate, minimally at test initiation (before organisms added), at 48 hrs and at end of test Static-renewal: each replicate, at test initiation and end, and just before and after each renewal Flow-through: each replicate at 0, 48, and 96 hrs, and every 96 hrs thereafter 	<ul style="list-style-type: none"> Concentration measured for each replicate at test initiation, 48 hours and 96 hours (end of test).

Guideline Criteria	Reported Information
Controls <ul style="list-style-type: none"> Consist of same dilution water, conditions, procedures and test population Negative and/or solvent Maximum allowable mortality 10% (or 1 mortality if 7 to 10 fish used) for 96 hr period; 10% additional past 96 hrs. 	<ul style="list-style-type: none"> Negative Control: dilution water. Humic Acid Control: dilution water and humic acid. No mortality observed in any controls.
Replicates <ul style="list-style-type: none"> Two per test concentration Equal volume test solution and number test fish 	<ul style="list-style-type: none"> Two per test concentration. Equal volume test solution and number of test fish.
Test Organisms <ul style="list-style-type: none"> Minimum 20 fish exposed to each test concentration Equal number per test chamber Not fed during treatment period Randomly or impartially assigned to test vessels within 30 min of addition of test substance Biological observations and removal of dead fish made at 24, 48, 72, and 96 hrs 	<ul style="list-style-type: none"> 10/replicate (20 fish/test concentration). Equal number per test chamber. Not fed during treatment period. Fish were added two at a time to each aquaria until each aquaria contained two fish. This procedure was repeated until each aquaria contained ten fish. Observations reported every 24 hours.

REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in the report?	<ul style="list-style-type: none"> Yes
Name of test facilities, test dates and personnel reported?	<ul style="list-style-type: none"> Test dates and facilities reported but not personnel
Identification of test substance (including physicochemical characteristics) and purity provided?	<ul style="list-style-type: none"> Yes
Methods used in preparation of stock solutions and analysis of test concentrations described? Accuracy of method (i.e., detection limit and quantification limit) reported?	<ul style="list-style-type: none"> Yes
LC ₅₀ concentration-response curves, LC ₅₀ values, and associated 95% C.I. determined for 24, 48, 72, and 96 hrs? NOEL also reported?	<ul style="list-style-type: none"> Yes

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Guideline Criteria	Reported Information
Graph of concentration-mortality curve at test termination and any control mortality observed during acclimation or study period provided?	<ul style="list-style-type: none"> Not provided
Any protocol deviations which may have influenced final results of test reported?	<ul style="list-style-type: none"> Yes
Raw data included?	<ul style="list-style-type: none"> Yes (in Appendices)
Signs of abnormal behavior by test fish (if any) described?	<ul style="list-style-type: none"> Not reported
Statistical methods reported?	<ul style="list-style-type: none"> Yes

Dose Response: DDAC (clean dilution water):

Nominal Concentration (mg ai/L)	Mean Measured Concentration (mg ai/L)	Number of Fish at Test Initiation	No. Dead Fish			
			24 hour	48 hour	72 hour	96 hour
Control	Control	20	0	0	0	0
0.065	0.054	20	0	0	0	0
0.11	0.092	20	0	0	0	0
0.18	0.16	20	0	3	3	3
0.30	0.27	20	18	19	19	20
0.50	0.46	20	20	20	20	20

Dose Response: DDAC with 10 mg/L Humic Acid:

Nominal Concentration (mg ai/L)	Mean Measured Concentration (mg ai/L)	Number of Fish at Test Initiation	No. Dead Fish			
			24 hour	48 hour	72 hour	96 hour
Negative Control	Negative Control	20	0	0	0	0
Humic Acid Control	Humic Acid Control	20	0	0	0	0
0.39	0.40	20	0	0	0	0
0.65	0.65	20	0	2	2	2
1.1	1.0	20	20	20	20	20
1.8	1.6	20	20	20	20	20
3.0	2.6	20	20	20	20	20

Dose Response: DDAC with 20 mg/L of Humic Acid:

Nominal Concentration (mg ai/L)	Mean Measured Concentration (mg ai/L)	Number of Fish at Test Initiation	No. Dead Fish			
			24 hour	48 hour	72 hour	96 hour
Negative Control	Negative Control	20	0	0	0	0
Humic Acid Control	Humic Acid Control	20	0	0	0	0
0.39	0.35	20	0	0	0	0
0.65	0.51	20	0	0	0	0
1.1	0.94	20	0	0	0	0
1.8	1.6	20	20	20	20	20
3.0	2.5	20	20	20	20	20

Statistical Results: The mean measured concentrations and corresponding mortality data were used to estimate the 24-, 48-, 72- and 96-hour median lethal concentrations (LC₅₀) and 95% confidence intervals based on Stephan (1982).

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DDAC (clean dilution water):

Duration	LC₅₀ (mg a.i./L)	95% Upper CI	95% Lower CI
24-hr	0.22	0.27	0.16
48-hr	0.20	0.27	0.16
72-hr	0.20	0.27	0.16
96-hr	0.19	0.27	0.16

NOEC through 96 hours = 0.092 mg a.i./L

DDAC with 10 mg/L of Humic Acid:

Duration	LC₅₀ (mg a.i./L)	95% Upper CI	95% Lower CI
24-hr	0.81	1.0	0.65
48-hr	0.77	1.0	0.65
72-hr	0.77	1.0	0.65
96-hr	0.77	1.0	0.65

NOEC through 96 hours = 0.40 mg a.i./L

DDAC with 20 mg/L of Humic Acid:

Duration	LC₅₀ (mg a.i./L)	95% Upper CI	95% Lower CI
24-hr	1.2	1.6	0.94
48-hr	1.2	1.6	0.94
72-hr	1.2	1.6	0.94
96-hr	1.2	1.6	0.94

NOEC through 96 hours = 0.94 mg a.i./L

VERIFICATION OF STATISTICAL RESULTS:

Method: EPA TOXANAL Program

DDAC (clean dilution water):

96-hr LC₅₀: 0.19 a.i. mg/L 95% C.I: 0.16 to 0.27 a.i. mg/L NOEC: 0.092 a.i. mg/L

```

C:\WINDOWS\system32\cmd.exe
  2.00      200      200      1000      95.00000000  95
  1.00      100      100      1000      95.00000000  95
  1.00      100      100      1000      95.00000000  95
  0.50      100      100      1000      95.00000000  95
  0.50      100      100      1000      95.00000000  95

THE BINOMIAL TEST SHOWS THAT 1.0 AND 2.0 CAN BE
USED AS SIGNIFICANTLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS. BECAUSE THE ACTUAL CONFIDENCE LIMIT
OBTAINED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LOAD FOR THIS SET OF DATA IS 0.40 MG/L.

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT RESPONSE IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE
PROFIT METHOD CAN GIVE ANY SIGNIFICANTLY SOUND RESULTS.

*****
DO YOU WISH TO RUN ANOTHER DATA SET?
ENTER Y OR N.
Y

```

DDAC with 10 mg/L of Humic Acid:96-hr LC₅₀: 0.80 a.i. mg/L 95% C.I: 0.65 to 1.0 a.i. mg/L NOEC: 0.40 a.i. mg/L

```

C:\WINDOWS\system32\cmd.exe
  2.00      200      200      1000      95.00000000  95
  1.00      100      100      1000      95.00000000  95
  1.00      100      100      1000      95.00000000  95
  0.50      100      100      1000      95.00000000  95
  0.50      100      100      1000      95.00000000  95

THE BINOMIAL TEST SHOWS THAT 1.0 AND 1.0 CAN BE
USED AS SIGNIFICANTLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS. BECAUSE THE ACTUAL CONFIDENCE LIMIT
OBTAINED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LOAD FOR THIS SET OF DATA IS 0.40 MG/L.

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT RESPONSE IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE
PROFIT METHOD CAN GIVE ANY SIGNIFICANTLY SOUND RESULTS.

*****
DO YOU WISH TO RUN ANOTHER DATA SET?
ENTER Y OR N.
Y

```

DDAC with 20 mg/L of Humic Acid:96-hr LC₅₀: 1.23 a.i. mg/L 95% C.I: 0.94 to 1.6 a.i. mg/L NOEC: 0.94 a.i. mg/L

C:\WINDOWS\system32\cmd.exe

	EXPOSED	DEAD	DEAD	PROTECTANT
1.0	0%	0%	0%	0%
1.0	0%	0%	0%	0%
1.0	0%	0%	0%	0%
1.0	0%	0%	0%	0%
1.0	0%	0%	0%	0%

THE REPORTED TEST SHOWS THAT THE END OF THE CONCENTRATION RANGE FOR THE DDAC CONCENTRATION IS 100% DEATH. BECAUSE THE DDAC CONCENTRATION IS 100% DEATH, THE DDAC CONCENTRATION IS 100% DEATH.

AN APPROPRIATE CONCENTRATION FOR THE DDAC CONCENTRATION IS 100% DEATH.

WHEN THERE ARE LESS THAN 100 CONCENTRATIONS OF DDAC, THE DDAC CONCENTRATION IS 100% DEATH. NEITHER THE DDAC CONCENTRATION NOR THE DDAC CONCENTRATION CAN GIVE ANY EFFECTS TO THE DDAC CONCENTRATION.

DO YOU WANT TO RUN ANOTHER TEST? (Y/N)

ENTER YOUR N.

CONCLUSIONS:

DDAC alone is highly toxic to the fathead minnow. DDAC combined with 10 to 20 mg/L humic acid reduces toxicity 4- to 6-fold. However, even when combined with humic acid, DDAC remains moderately to highly toxic to fathead minnows. Most reported mortalities occurred within the first 24 hours of exposure.

The report states that the study was conducted in a static rather than a flow through system "... because of difficulties encountered by the Study sponsor in attempting to conduct flow through aquatic toxicity studies with other similar quaternary ammonium compounds (i.e., didecyldimethylammonium chloride (DDAC)). these difficulties were associated with the inherent strong adsorptive properties of the chemicals plus the low concentrations which have to be evaluated."

Sign-off Date : 10/21/09

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